# STUDY OF THE EFFECT OF FERTILIZERS AND GROWTH REGULATORS ON THE GROWTH AND REPRODUCTION IN *MORINGA OLEIFERA* LAM. AND ITS RELATIONSHIP WITH THE PURIFICATION OF THE POLLUTED WATER BY THE PLANT EXTRACTS

## INTRODUCTION

*Moringa oleifera* Lam. is a perennial small tree belonging to Moringaceae. The plant is highly medicinal and all parts of it are used as remedy for several diseases. It has three varieties, wild, PKM-1 and PKM-2. The wild variety did not require application of fertilizers. PKM-1 and PKM-2 need addition of fertilizers. Addition of fertilizers changes the soil conditions, which in turn can change the biochemical principles quantitatively and qualitatively. In the present study, inorganic and organic fertilizers and biofertilizer Azospirillum were added singly or in combinations. The change in soil qualities was studied. The growth, flowering and fruiting were analysed using the data obtained.

Among the various properties, one important quality observed in *M. oleifera* is the coagulation properties and antimicrobial effects of the seed powder prepared from ripened seeds. Now almost all the sources of water became polluted by different causes, such as waste water releasing from industries, leaching of chemical fertilizers used in the field, washing out of animal and domestic sewage from human inhabitations, use of ponds and rivers as laundries, etc. Chemical purification of polluted water is reported to have health problems, such as cancer, Alzheimer's disease, etc. among the people (Ghebremichael *et al*., 2005; Muyibi and Alfugura, 2003). Natural coagulants can substitute the chemicals, of which *M. oleifera* seed powder was observed to be most efficient, and readily available (Amagloh and Benang, 2009). A major component in the polluted water is bacteria. Hence, the antibacterial effect of the seed powder was investigated. Further, study was also done regarding the major biochemical principle involved in the water purification and antimicrobial effect. In all the previous investigations regarding the purification of natural waters as well as industrial effluents

and antibacterial studies the wild Moringa seed powder was used. Practically no study was reported in the new variety, PKM-1, widely cultivated in Tamil Nadu and certain regions of Kerala. Hence, the present study was conducted with the following objectives.

## OBJECTIVES OF THE STUDY

i) A systematic evaluation of the effect of organic/ inorganic/ biological fertilizers on growth and yield of PKM-1 variety of *Moringa oleifera*.

ii) To find out the effect of foliar spray of growth regulators in flowering and fruiting of *M. oleifera* PKM-1 variety.

iii) To study the water purifying effect of the seed powder extracts of PKM-1 (standard and fertilizer treated) and wild variety of *Moringa oleifera* in various solvents like distilled water, 1% NaCl, 0.1N NaOH, and 70% ethanol.

iv) To investigate the antibacterial property of PKM-1 Moringa variety since bacteria is one of main components of polluted water.

v) To identify and study the major biochemical components responsible for the water purifying and antibacterial ability of *M. oleifera*.

## METHODOLOGY EMPLOYED

To achieve the objectives suitable methods were employed.

### 1. Cultivation of Moringa plants with the application of fertilizers and growth regulators.

The standard PKM-1 seeds purchased from Periakulum centre of Tamil Nadu Agricultural University were germinated in polybags containing a mixture of Red soil, sand and dried powdered cow dung in 2:2:1 proportion. In the experiments using Azospirillum, the bacteria were inoculated to the soil along with the seeds in the polybags on which the combination of fertilizer application was labelled. The grown up seedlings were transplanted to the field. The transplantation was done in randomized block design (RBD) in triplicates. The fertilizers, namely, NPK and vermicompost were added alone or in combination, after the establishment of seedlings in the field. The plants were

ratooned after the harvest in the first year. The organic and inorganic fertilizers were applied after ratooning as in first year. The mature and dried seeds were collected and used for the analytical procedures of water purification, antibacterial studies and identification of biochemical components.

The foliar spray of flowering hormones $GA_3$ and ethaphone was done before the onset of flowering in both years and the effect of foliar spray was studied by comparing with control plants.

## 2. Analysis of the effect of fertilizers on soil qualities and role of fertilizers and growth regulators on growth and yield of *M. oleifera* PKM-1 plants.

The qualitative and quantitative analysis of soil was done after 30, 60, 90 and 120 days of the application of NPK and vermicompost, by collecting the soil at 3-4 inches depth from the base of plants. The soil parameters such as, soil pH, electric conductivity, percentage of organic carbon, available phosphorus, potassium and micronutrients like Fe, Cu, Mn and Zn were measured using standard methods.

The height, girth and number of branches were measured and tabulated before transplantation and in one month interval till flowering after transplantation and fertilizer treatment. The data on the number of branches, inflorescences and fruits was collected in the second year also.

## 3. Isolation and enumeration of Azospirillum in the rhizosphere soil

Soil samples were collected from the rhizosphere soil after 30, 60, 90 and 120 days of application of vermicompost and NPK. The Azospirillum cells were isolated by serial dilution method (Aneja, 2003) and cultured in petri plates in triplicate containing RC (Rojo Congo) medium, according to the SPC technique suggested by Aneja (2003). After 72 hours the plates were observed for the number of Azospirillum colonies in each dilution using colony counter.

The root tissue also was analysed for the presence of internal root colonization of bacteria, according to the method described by Dobereiner *et al.* (1976).

## 4. Purification of well and pond waters by the seed extracts of Moringa varieties

Yellowish coloured well water was collected from a well at Thrissur District near Chavakkad sea shore and pond water was obtained from Chattukulam, a highly polluted pond near Guruvayoor at Thrissur District. After preliminary tests, the experiments were designed with selected concentrations of 100, 150 and 200mgl$^{-1}$ extracts of seed powder in four different solvents, according to the procedure given by Harborne (1973). The seeds selected were that of *M. oleifera* PKM-1 variety purchased from Periakulam, the seeds obtained from the cultivated plants near L. F. College campus by using $A_4$+V+N combinations of fertilizers and that of wild Moringa growing near Kunnamkulam, Thrissur district.

The seeds were powdered and defatted using soxhlet apparatus. Defatted seed powder of three types of Moringa was weighed and extracted with 5ml each of different solvents such as distilled water, 1%NaCl, 0.1 N NaOH and 70% ethanol and the supernatants were directly added to the measured volume of the polluted water samples, stirred with magnetic stirrer at 500 rpm and kept still to settle down. After three hours the supernatants of the settled waters were collected and stored at $4^0$C in a refrigerator for further analysis.

The analysis of water samples and their non-treated controls was done by using various parameters such as turbidity, pH, electric conductivity(EC), total dissolved solids (TDS), salinity, total alkalinity, total hardness, calcium hardness (Ca.H), magnesium hardness (Mg.H), chloride ($Cl^-$), fluoride ($F^-$), Ammonium ($NH_4$-N), phosphate ($PO_4$ –P), sulphate ($SO_4$-S), nitrate ($NO_3$-N), dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD) by standard methods.

## 5. Purification industrial waste waters by *M. oleifera* seed extracts

Dairy industry waste water (DIWW) was collected from Malanad developmental Society, Kanjirappally, Kottayam District, and Rubber factory effluent (RFE) was obtained from Neerackal latex private limited, Kuravilangad, Kottayam District. The treatments were done after ten times dilution of the effluent with distilled water. The results were tabulated by multiplying with dilution factor. After initial screening tests, the experiments were designed with the extracts containing 1-2 $gl^{-1}$ seed powder of PKM-1, PKM-1($A_4$+V+N) and wild variety, on both industrial effluents. The extracts of $1gl^{-1}$, $1.5gl^{-1}$ and $2gl^{-1}$ defatted seed powder of all the varieties were prepared in 10ml each of distilled water, 1% NaCl, 0.1N NaOH and 70% ethanol and centrifuged. The supernatants were added to the measured volume of industrial effluents, agitated using a magnetic stirrer at 500 rpm for 5min. and kept for 3 hour and to overnight. The treated effluents were filtered through simple sand filters and the filtrates were collected and stored at $4^0$C in the refrigerator for further analysis. The parameters tested for natural waters were selected for testing the industrial effluents also, except DO.

## 6. Treatment of RFE with *M. oleifera* seed powder for the removal of heavy metals

On screening of the four types of polluted waters, heavy metal content such as chromium, Iron, Zinc and Manganese was detected only in the rubber factory effluent. The NaCl extract was selected to treat RFE for heavy metal analysis. The non-treated and treated samples were tested for the content of heavy metals by ICP-AES (Inductively coupled plasma atomic emission spectrometry) analysis.

## 7. *In vitro* study of bacterial count in *M. oleifera* seed extract treated water samples and the antibacterial effect of the extracts in the bacterial cultures.

The non-treated polluted water samples and the water samples after treatment with PKM-1and wild Moringa seed extracts were analysed for the presence of bacteria. Standard plate count technique described by Aneja (2003) was adopted for the *in vitro* culturing of bacteria for assessing the bacterial count in water samples.

The non-defatted and defatted seed powders of PKM-1and wild Moringa as well as *in vitro* produced seed callus were used for the antibacterial studies. The extracts of the seed and callus powder in three different concentrations (100, 150 and 200mg) were prepared in 10 ml each of distilled water, 1% NaCl, 0,1N NaOH and 70% ethanol. The antibacterial activity was screened by disc diffusion method (Barry and Thornberry, 1991) on four species of bacteria such as *Bacillus cereus, E. coli, Salmonella typhii* and *Vibrio cholarae*, the human pathogens causing serious water borne diseases. Since the NaOH extract and callus extracts were found ineffective, only water and NaCl extracts of non-defatted and defatted seed powders were used for further studies. The analysis was repeated with these extracts in triplicates by keeping discs with pure solvents as positive control, and ciprofloxacin disc as negative control along with other discs in each petri plate.

**8. Phytochemical analysis of *M. oleifera* seed powder**

Estimation of the total proteins and the proteins dissolved in the four solvents like distilled water, 1% NaCl, 0.1N NaOH and 70% ethanol of PKM-1, PKM-1($A_4$+V+N) and wild Moringa were done by Lowry's method (Lowry *et al.*, 1951). Also other important components of the seeds such as carbohydrates, lipids, free amino acids, phenol, tannins and ascorbic acid (vitamin-C) were estimated using standard methods (Sadasivam and Manikam, 1997).

The water and NaCl extracts were further purified by ammonium sulphate precipitation and fractionation. The purified fractions were tested by coagulation activity assay using synthetic turbid water and the better performed fraction was selected for gel electrophoretic separation using SDS-PAGE in order to find out the molecular weight of the fraction.

## RESULT AND DISCUSSION

### 1. Effect of fertilizers on the chemical characteristics of the soil and growth and yield of Moringa plants.

On analyzing the different chemical characteristics of the soil, such as electric conductivity, percentage of organic carbon, available phosphorus, potassium and micronutrients, the combined effect of fertilizers was found to increase these components than the single manure treated soils. Among the combined treatments, $10kgh^{-1}$ Azospirillum with $5000kgh^{-1}$ vermicompost and $125kgh^{-1}$ NPK designated as $A_4$+V+N (Azospirillum, vermicompost and NPK) combination of fertilizers gave best having maximum enhancement of EC, macro and micro nutrients. The pH was found to be lowered in all the vermicompost treated samples both in single and combined treatments. Azospirillum treated soil also was showed slightly lower pH. Among the single manure treated soils vermicompost alone treated soils exhibited long lasting effects. When the number of Azospirillum in the soil was enumerated maximum number was observed in the soil treated with A4+V+N fertilizer combination.

Vermicompost as an organic matter decreases the pH because of the presence of organic acids such as amino acids, fatty acids, etc. by the degradation of proteins, fats, etc. (Azarmi *et al*., 2008). Magdi *et al*. (2011) reported that the combined effect of fertilizers gave best quality soil. The reason may be that the decomposition of vermicompost and other organic manures is increased by the presence of Azospirillum and other biofertilizers. The presence of small quantities inorganic fertilizer acts as a stimulant for the bacteria to grow and establish, which in turn increases the fertility and thus the physical conditions of the soil become conducive for the growth of Azospirillum and thus the soil become more and more fertile (Aishah *et al*., 2009). Bhardwaj and Gaur (1970) also reported the presence of nitrogen fixing bacteria such as Azospirillum improves soil quality and nutrient conditions of the soil by nitrogen fixation and decomposing the organic matter in the organic manure and soil.

A similar observation was noticed in the growth and yield of *M. oleifera* plants. The seeds treated with $10kgh^{-1}$ Azospirillum exhibited early sprouting, maximum percentage of germination and survival. The maximum growth and number of fruits was for the plants treated with $A_4$+V+N combination of fertilizers. Next higher yield was observed for plants treated with $A_2$+V+N and $A_4$+V combination. The maximum available nutrients make the soil conditions conducive for better growth and yield in the plants. The present observation is in tune with the findings of many investigators, that the combined effect of inorganic, organic and biofertilizers gave a maximum growth and yield in different species of crop plants (Krishnamoorthy *et al.*1995; Mulani *et al.* 2007).

## 2. Effect of seed powder extracts of PKM-1, PKM-1 ($A_4$+V+N) and wild Moringa in the purification of polluted waters

Almost all the extracts showed a certain quantity of removal of pollutants from the polluted waters. An important observation was that the seeds collected from the cultivated PKM-1 plants exhibited similar activity as that of the purchased standard PKM-1 seeds. It shows that the applied fertilizers did not affect the quality or quantity of the components of the seeds, which are responsible for water purification. The better removal of different components of the polluted waters was achieved by various extracts in various concentrations. Of which NaCl extracts effected the removal of more number of components in all the types of polluted waters.

With regard to well and pond water, NaCl extracts in various concentrations gave maximum removal of pollutant components. Water extracts and ethanol extracts also exhibited better removal of some components. When the industrial effluents, DIWW and RFE are considered, a similar observation was noticed, in which the $2gl^{-1}$ NaCl extract gave highest percentage of removal of the pollutants. PKM-1 variety performed better than the wild Moringa seed extracts.

As a whole NaCl extracts of PKM-1 seeds are found to be more efficient in removing pollutants from the different types of polluted waters. Certain reports are

available regarding water purification property of plant products. Jahn (1986) reported that 200mgl$^{-1}$ *M. oleifera* seed powder extract is effective in water clarification. According to Folkard *et al.* (1999) *M. oleifera* dosed at 150mgl$^{-1}$ water extract gave a removal of 40% BOD and COD and 80% suspended solids. Folkard and Sutherland (2001) further observed that extraction of proteins using 1M sodium chloride solution gave enhanced coagulation at significantly reduced concentrations compared to water extracted material. Amagloh and Benang (2009) found that increasing concentrations of *M. oleifera* seed extract treatment led to decrease in conductivity values.

In the present investigation very high efficiency was noticed in removing the heavy metals, chromium, Iron, and Zinc from RFE by NaCl extract treatment. The removal was found to be increased with increase in concentration of the seed powder in the extracts.

## 3. Antibacterial efficiency of *M. oleifera* seed powder

Among the various treatments, the polluted water treated with 200mgl$^{-1}$ defatted seed powder extracts of *M. oleifera* PKM-1 in 1% NaCl was exhibiting the maximum inhibition in all the four types of bacterial cultures. A gradual increase in size of inhibition zone was noticed for all extracts with increasing concentration of the seed powder. Out of the four bacterial cultures, E. coli was showing maximum inhibition with NaCl extract of 200mg defatted PKM-1 of seed powder. i.e. 27.5 mm. diameter.

Antibacterial property of seed extract of *M. oleifera* is claimed by Foidl *et al.* (2001). Kawo and Daneji (2011) reported high level of bacterial removal from raw waters by using water extracts of wild Moringa.

## 4. Phytochemical Components of *M. oleifera* seed powder effecting water purification and antibacterial activity.

Since *M. oleifera* seed powder demonstrated water purifying ability further study was done regarding the biochemical principle behind it. The total protein content and the

protein content in various extracts were analysed and found that the water extract contain the highest content of proteins and NaCl, NaOH and ethanol extracts contain lesser amount than water extract in a descending order. But as the proteins in the NaCl extract were found to be active than water extract, further phytochemical analysis was done only with NaCl extract. In the ammonium sulphate fractionation and purification efficiency assay 60% fraction of NaCl extract of PKM-1 types and wild varieties of Moringa seed powder was found to be better than the 40% and 80% fractions. Hence, 60% fraction of NaCl extract of PKM-1, PKM-1($A_4$+V+N) and wild varieties of Moringa seed powder was undertaken for isolation and molecular weight determination by gel electrophoresis using SDS-PAGE method. A thick band at about 13.4 KDa position of the marker protein was separated.

Sahni *et al.* (2008) fractionated five prominent cationic protein bands from Moringa seed extract, of which the fraction having molecular weight in the range 6-16 KDa, was the prominent one and they suggested that it might be playing major role in removing arsenic from water. Ndabigengesere, *et al*. (1995) described dimeric cationic proteins with 12-14 Kda molecular mass. They have the opinion that the active protein is actually composed of two 6.5 Kda subunits connected with an S-S bond, that is cleaved when protein extraction occurs in reducing conditions. Kwaambwa & Maikokera (2008) also separated a protein having a molecular weight 12-14 after ammonium sulphate precipitation and column chromatography. The findings of these investigators support the present observation that the most effective protein in the purification of polluted water is the one with a molecular weight of around 13.4 KDa. According to Schwarz (2000) the seed kernel of M. *oleifera* contains lower molecular weight soluble proteins which carry positive charge. When it is added to polluted water, they act as magnets and attracting the prominently negative charged particles. Under proper agitation these bound particles grow in size in the form of flocs, which are left to settle by gravity.

## CONCLUSIONS

1. A combination of fertilizers containing 10Kgh$^{-1}$ Azospirillum, 5000Kgh$^{-1}$ vermicompost and 125Kgh$^{-1}$ NPK is most effective in enhancing soil quality and thereby increasing the growth and yield of *M. oleifera* plants.
2. Change of fertilizers does not affect the quality and quantity of active components/ gm of the seed and hence, PKM-1 and PKM-1($A_4$+V+N) are showing only slight difference in their activity in water purification and antibacterial efficiency.
3. The foliar spray of growth regulators have no effect on flowering and fruiting of *M. oleifera*.
4. The NaCl extracts especially higher concentrations of the seed powder is more efficient in removing most of the components of the polluted waters.
5. The PKM-1 variety is more efficient than wild variety in its water purification.
6. The defatted seed powder extract is more effective in antibacterial quality than non-defatted seed powder.
7. Polypeptides/proteins around molecular weight of 13.4 KDa is detected as the most effective active component responsible for water purification.

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